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A CAPSAICIN-SENSITIVE INHIBITORY REFLEX FROM THE COLON TO MESENTERIC ARTERIES IN THE GUINEA-PIG

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SUMMARY

1. The present *in vitro* study examined the effect of distension of the distal colon on membrane potential in the inferior mesenteric artery of the guinea-pig.

2. Distension of the distal colon up to an intraluminal pressure of 25 cmH_2O induced a hyperpolarization in the inferior mesenteric artery. The average amplitude of hyperpolarizations induced by 2 min distensions of the colon was 3 mV and their average duration was 268 s.

3. Distension-induced hyperpolarizations (DIHs) were abolished in the presence of tetrodotoxin or a low-Ca^{2+} (0·5 mM) superfusion solution.

4. Superfusion of capsaicin (10 μM) induced slow hyperpolarizing responses in mesenteric arteries. Following application of capsaicin (10 μM), DIHs were abolished.

5. These findings provide strong evidence that mesenteric arteries receive an inhibitory, capsaicin-sensitive sensory innervation from the distal colon which is activated during periods of colon distension to induce hyperpolarization of the arterial smooth muscle. This extramural inhibitory reflex pathway may play a physiological role in co-ordinating mesenteric blood flow with changes in gut motility.

INTRODUCTION

The activation of primary afferent sensory nerve endings by mechanical injury, chemicals, or electrical stimulation produces localized vasodilatation through a presumed axon reflex (Bayliss, 1901; Langley, 1923; Lewis, 1937). The physiological role of primary afferent nerves in the regulation of vascular function is not known. In previous *in vitro* studies in the guinea-pig inferior mesenteric artery (Kreulen, 1986; Meehan, Hottenstein & Kreulen, 1991), we demonstrated that low-frequency (0·5–5 Hz) nerve stimulation elicited slow inhibitory junction potentials and dilatation through the activation of capsaicin-sensitive periarterial nerves. On the basis of these findings, we hypothesized that these periarterial capsaicin-sensitive nerves may be primary afferent fibres whose peripheral endings are located in the distal colon, and which are activated during mechanical stimulation of the gut (Meehan *et al.* 1991). The present study was designed to test this hypothesis. Intracellular electrical responses in the guinea-pig inferior mesenteric artery to distension of an attached segment of the distal colon, were examined. We provide

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evidence that capsaicin-sensitive nerves mediate an inhibitory distension-induced slow hyperpolarization (DIH) in the inferior mesenteric artery. We propose that mesenteric arteries receive an inhibitory capsaicin-sensitive input from the gut which is activated during gut distension to produce hyperpolarization and vasodilatation.

METHODS

Guinea-pigs of either sex weighing 150–250 g were used. Animals were killed by cervical dislocation and exsanguinated. The isolated mesocolon preparation used in the present study has been described previously (Crowcroft, Holman & Szurszewski, 1971; Kreulen, 1986). Briefly, a section of mesentery containing a distal segment of the inferior mesenteric artery (diameter: 150–300 μm), together with an attached segment of the distal colon, was excised, mounted in a recording chamber, and superfused with oxygenated Krebs solution (37 °C). In the present study, the inferior mesenteric ganglion was removed from the mesocolon preparation. Small pins were used to secure the mesentery adjoining the inferior mesenteric artery and the colon segment to the Sylgard bottom of the recording chamber, thus preventing movement of the artery during colon distension.

Colon distension

The orad end of a 2 cm-long colonic segment was ligated and the caudad end of the segment was cannulated with a glass tube which was attached to a calibrated vertical manometer and a pressure transducer. This distending apparatus allows the monitoring of intraluminal pressure as well as enabling the distension of the colon segment with Krebs solution.

Electrophysiological measurements

Intracellular recordings of membrane potential in arteries were made using glass filament microelectrodes filled with 3 M-KCl and having tip resistances ranging from 60 to 100 MΩ. Membrane potentials and intracolonic pressure measurements were recorded on analog tape and reproduced for figures using a Gould *X–Y* plotter or a Gould (Cleveland, OH, USA) brush pen-recorder.

Drugs and solutions

The composition of the Krebs solution was (mM): NaCl, 117; KCl, 4·7; $CaCl_2$, 2·5; $MgCl_2$, 1·2; $NaHCO_3$, 25; NaH_2PO_4, 1·2, and D-(−)-glucose, 11·5. The Krebs solutions in supply reservoirs were gassed continuously with a mixture of 5% CO_2 in O_2. The drugs used were as follows: capsaicin (ICN Biochemicals, Cleveland, OH, USA); tetrodotoxin (TTX; Calbiochem, San Diego, CA, USA). Drugs were applied by addition to the Krebs superfusion solution in the required concentrations.

RESULTS

Electrical responses to colon distension in the inferior mesenteric artery

The mean resting membrane potential of artery cells was $-65 \pm 1{\cdot}7$ mV ($n = 35$, twelve preparations). In twenty-nine out of the thirty-five artery cells tested, slow hyperpolarizing responses were elicited by a 2 min distension of the attached colon segment (distending pressure, 25 cmH_2O). Figure 1 shows a distension-induced slow hyperpolarization (DIH) in an artery cell. The onsets of DIHs occurred usually 20–60 s after the onset of colon distension. The average amplitude of DIHs was $3 \pm 0{\cdot}3$ mV and their average duration was 268 ± 33 s ($n = 29$). In five control cells (four preparations), there was no time-dependent change in the amplitudes of DIHs over the duration of the experiment, when colon distension was induced at 5 min intervals.

In order to test whether the DIH was neuronally mediated, the effects of tetrodotoxin and of a low-Ca^{2+} superfusion solution were tested. Figure 2 shows the effect of tetrodotoxin (1 μM) on a DIH in an artery cell. Tetrodotoxin (1 μM) had no effect on resting membrane potential; in the presence of tetrodotoxin, DIHs were

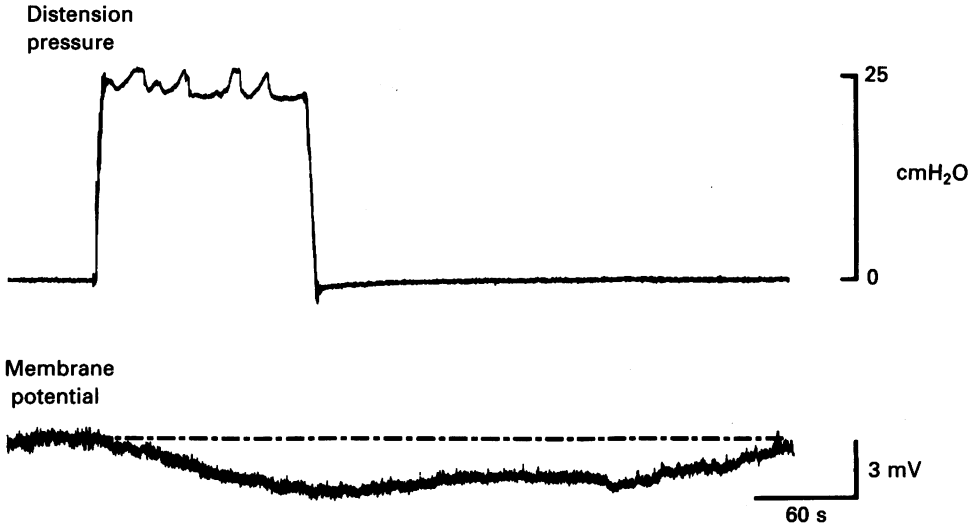

Fig. 1. Effect of colon distension on membrane potential in a mesenteric artery of the guinea-pig. A 2 min distension at 25 cmH_2O induced a 3 mV hyperpolarization which lasted for 377 s. Resting cell membrane potential, −63 mV.

abolished in mesenteric arteries (four cells, three preparations). Superfusion of a low-Ca^{2+} (0·5 mM) solution had no effect on membrane potential; however, in the presence of a low-Ca^{2+} superfusion solution, DIHs were abolished (three cells, three preparations).

Capsaicin

The involvement of capsaicin-sensitive nerves in the arterial DIH was examined by addition of capsaicin, at the concentration of 10 μM, to the superfusion solution. Application of capsaicin induced a hyperpolarizing response which was transient, lasting about 2 min (Fig. 3). Presumably, capsaicin induced the release of a transmitter(s) from periarterial peptidergic nerves, resulting in hyperpolarization of the vascular smooth muscle (Buck & Burks, 1986; Meehan *et al.* 1991). The mean amplitude of capsaicin-induced hyperpolarizations in mesenteric arteries was $3 \pm 0{\cdot}7$ mV (six cells, six preparations). As shown in Fig. 3, following application of capsaicin (10 μM), DIHs were abolished. In three preparations in which experiments of the kind shown in Fig. 3 were made, the mean amplitude of DIHs obtained prior to capsaicin treatment was 3 ± 1 mV (three cells). Following application of capsaicin (10 μM), DIHs, as well as hyperpolarizing effects of subsequent applications of capsaicin (10 μM), were abolished (six cells tested; three preparations).

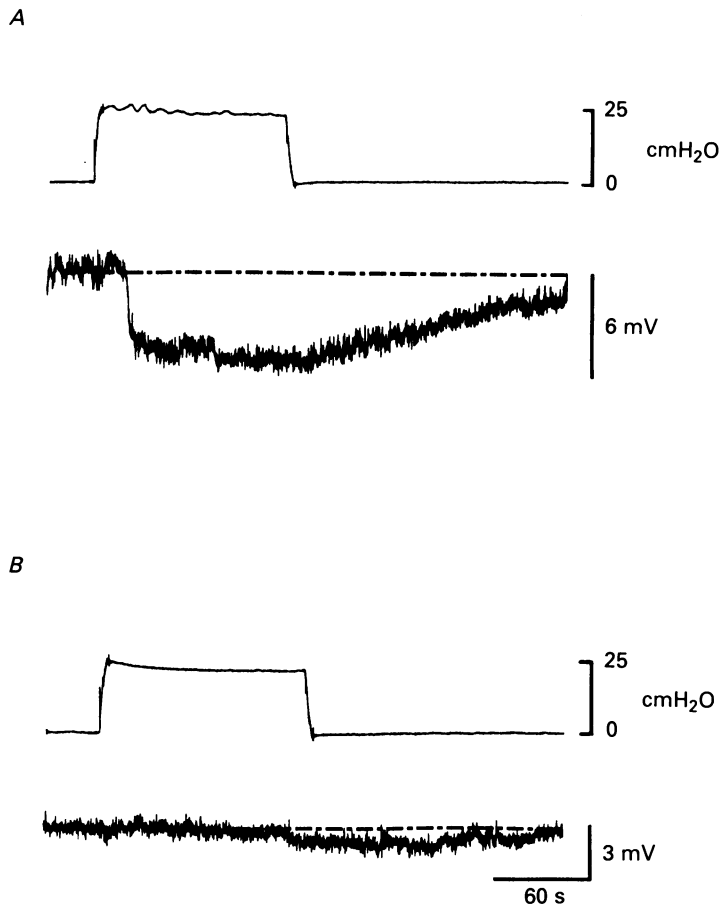

Fig. 2. Effect of tetrodotoxin on the distension-induced slow hyperpolarization in a mesenteric artery of the guinea-pig. *A*, a 2 min control distension of the colon elicited a 6 mV slow hyperpolarization in the mesenteric artery. *B*, tetrodotoxin (1 μM) abolished the distension-induced hyperpolarization. Resting cell membrane potential, −65 mV.

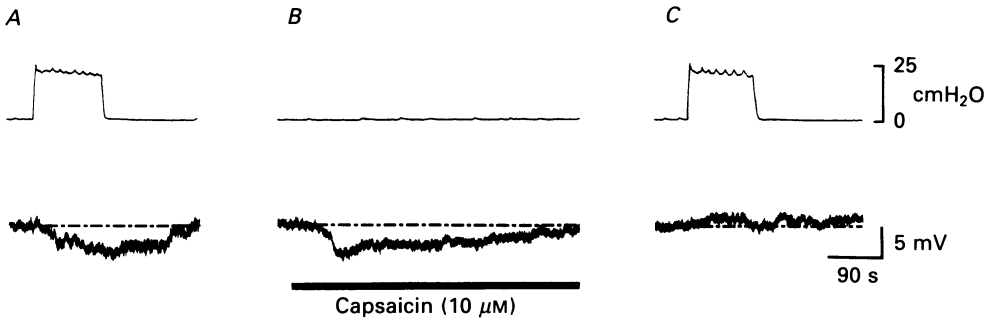

Fig. 3. Effects of capsaicin on membrane potential and on the distension-induced slow hyperpolarization in a mesenteric artery of the guinea-pig. *A*, a 2 min distension of the distal colon induced a 3 mV slow arterial hyperpolarization. *B*, superfusion of capsaicin (10 μM) evoked a 5 mV slow hyperpolarizing response. *C*, following capsaicin, the distension-induced hyperpolarization was abolished. Resting cell membrane potential, −66 mV.

DISCUSSION

The present study demonstrates the existence of a sensory pathway from the distal colon to the inferior mesenteric artery which, when activated by colon distension, produces capsaicin-sensitive hyperpolarizations in mesenteric arteries. Our study is the first report of a hyperpolarizing response to a physiological stimulus, as opposed to electrical nerve stimulation, in mesenteric arteries. In previous studies using the guinea-pig inferior mesenteric artery (Kreulen, 1986; Meehan *et al.* 1991), we demonstrated that electrical stimulation of perivascular capsaicin-sensitive nerves elicited slow inhibitory junction potentials, which were accompanied by dilator responses. In the light of these previous findings, we proposed that the DIH may underlie smooth muscle relaxation in guinea-pig mesenteric arteries.

DIHs were prevented by tetrodotoxin and by a low-Ca^{2+} superfusion solution, indicating that they were mediated by the activation of periarterial nerves, presumably resulting in the release of a transmitter(s) substance. The question arises regarding the type of sensory nerve which mediates the DIH in mesenteric arteries of the guinea-pig. In previous studies using the guinea-pig mesocolon preparation (Peters & Kreulen, 1984; Kreulen & Peters, 1986), we demonstrated the existence of a mechanosensory input from the colon to the inferior mesenteric ganglion which, when activated by colon distension, produced slow depolarizing responses in inferior mesenteric ganglion neurones. Slow depolarizing responses to colon distension were decreased by pre-treatment *in vivo* with capsaicin (Kreulen & Peters, 1986), suggesting that the slow depolarizations were mediated, at least in part, by capsaicin-sensitive dorsal root afferent neurones. Previous histochemical studies of peptide-containing nerves in the guinea-pig have provided evidence that some of the nerve fibres which pass from the colon to the adjacent mesenteric arteries have cell bodies in the dorsal root ganglia, while other neurones have their cell bodies in the gut wall (Furness, Papka, Della, Costa & Eskay, 1982; Dalsgaard, Hökfelt, Schultzberg, Lundberg, Terenius, Dockray & Goldstein, 1983). In view of these findings, it would seem reasonable to assume that the DIH may be mediated by either primary afferent nerves, or by sensory fibres whose cell bodies reside in the gut wall, or by both types of fibres. In the present study, 10 μM-capsaicin abolished the DIH in guinea-pig mesenteric arteries. In high concentrations (i.e. supramicromolar), capsaicin depletes the peptidergic transmitter stores and causes irreversible axonal damage in primary afferent nerve endings, thereby inhibiting neurotransmission in these nerves (Buck & Burks, 1986; Maggi & Meli, 1988; Bevan & Szolcsanyi, 1990). In contrast to its neurotoxic action in dorsal root C fibres, capsaicin has been shown to have no effect on the peptidergic transmitter stores of afferent fibres whose cell bodies reside in the colon (Furness *et al.* 1982). In view of the selectivity of capsaicin for primary afferent fibres, it appears likely that these nerves solely mediate the DIH in guinea-pig mesenteric arteries.

The present study provides the first report of a physiologically evoked axon reflex which involves mechanoreceptor-mediated activation of capsaicin-sensitive sensory nerve endings only. Previous studies which examined mechanoreceptor-mediated axon reflexes in the skin and viscera demonstrated that these reflexes were either partially sensitive or completely insensitive to capsaicin, indicating that afferent sensory nerves other than capsaicin-sensitive fibres were involved (Magi & Meli,

1988; Bevan and Szolcsanyi, 1990). Thus, the guinea-pig mesocolon preparation used in the present study may be a novel model for the study of the 'sensory-efferent function' of capsaicin-sensitive primary afferents (Maggi & Meli, 1988).

The inhibitory transmitter substance(s) mediating the arterial DIH is as yet unidentified. The peripheral sensory nerves which are activated by capsaicin have been shown to contain a variety of peptidergic transmitters, including: substance P, neurokinin A, vasoactive intestinal peptide and calcitonin gene-related peptide (CGRP) (Buck & Burks, 1986; Holzer, 1988). CGRP, in particular, may be a likely candidate as the mediator of the DIH. Thus CGRP has been shown to evoke both hyperpolarization and relaxation of mesenteric arteries through the activation of ATP-sensitive potassium channels in the smooth muscle cell membrane (Kawasaki, Takasaki, Saito & Goto, 1988; Nelson, Huang, Brayden, Hescheler & Standen, 1990; Han, Naes & Westfall, 1990).

In summary, the findings of the present *in vitro* study demonstrate that colon distension evokes slow hyperpolarizations and, presumably, relaxation in the inferior mesenteric artery of the guinea-pig, through the activation of mechanosensory inhibitory, capsaicin-sensitive afferent nerves. The mechanosensory input from the gut to mesenteric arteries may provide the basis for a local extramural reflex pathway involved in the co-ordination of mesenteric blood flow with gut motility. The *in vitro* preparation of the guinea-pig inferior mesenteric artery and colon may serve as a new model for the study of the mechanisms underlying the haemodynamic consequences of intestinal distension.

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